

Registration of DGE-3, a Durum Wheat Disomic Substitution Line 1E(1B) Involving a Wheatgrass Chromosome

Prem P. Jauhar* and Terrance S. Peterson

ABSTRACT

Durum wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB genomes) alien disomic substitution 1E(1B) line DGE-3 (Reg. No. GS-172, PI 665473) was developed by the USDA-ARS, Northern Crop Science Lab, Cereal Crops Research Unit, Fargo, ND and released in 2012. It was derived from the durum alien disomic addition line DGE-1 ($2n = 28 + 2$) having 28 durum chromosomes and the chromosome pair 1E of diploid wheatgrass, *Lophopyrum elongatum* (Host) Á. Löve. The pedigree of DGE-3 is PI 645483/Langdon' 1D(1B)*5. By crossing DGE-1 (PI 645483) with durum disomic substitution line 1D(1B), we produced hybrid derivatives with univalents of chromosomes 1B, 1D, and 1E. We selfed the hybrid derivatives five times and screened each generation for durum homoeologues 1A and 1B. We tested these hybrid derivatives for the presence of the alien chromosome 1E and for the absence of 1D. Using the chromosome-specific molecular marker *Xwmc333* for identifying chromosome 1A, *Xwgm18* for 1B, *Xwmc147* for 1D, and *Xedm17* for 1E, we isolated a durum disomic alien substitution line 1E(1B), which is fertile and stable and has been released as a new genetic stock, DGE-3. This substitution line has $2n = 28$ chromosomes that consist of 26 durum chromosomes plus the wheatgrass chromosome pair, 1E, which compensates for the missing 1B pair of Langdon. The disomic substitution forms 14 bivalents and is meiotically regular and fertile.

Durum wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB genomes) is a valuable cereal used for human consumption in the United States, Canada, and in several European countries. Although it is an allotetraploid with seven homoeologous groups of four chromosomes each, durum wheat can tolerate addition of related chromosomes as well as substitution of its chromosomes by those of related species. Thus, we developed a stable alien disomic addition line, DGE-1 (PI 645483; DGE, durum germplasm enhancement), with $2n = 28 + 2$ chromosomes, by adding to durum cultivar Langdon, chromosome 1E pair of diploid wheatgrass [*Lophopyrum elongatum* (Host) Á. Löve, $2n = 2x =$

14; EE genome] (Jauhar and Peterson, 2008; Jauhar et al., 2009). The *L. elongatum* accession (PI 531719) was obtained from the USDA-ARS, National Small Grains Collection, Aberdeen, ID. We have also been trying to substitute the homoeologous group-1 chromosomes of Langdon with a corresponding wheatgrass chromosome. By crossing DGE-1 with the Langdon alien substitution line 1D(1B) (Joppa, 1987), we produced a durum disomic alien substitution line 1E(1B) with $2n = 28$ chromosomes that consist of 13 pairs of durum chromosomes and the wheatgrass chromosome pair 1E. Thus, the alien chromosome pair 1E compensates for the absence of the native chromosome pair 1B of durum wheat. This new substitution line is cytologically stable and fertile and is being released as a new genetic stock, DGE-3 (Reg. No. GS-172, PI 665473). Its pedigree is DGE-1/Langdon 1D(1B)*5.

Materials and Methods

The durum disomic addition line DGE-1 and the disomic substitution line 1D(1B) were planted in 12.5-cm pots filled with Sunshine #1 soil mix (Sun Gro Horticulture, Ltd.) and grown in the greenhouse under a controlled temperature and lighting regime: 20–24°C and 16 h light/8 h dark cycle. In December 2006, we crossed DGE-1 and 1D(1B), using DGE-1 as the female parent. Crosses were made according to techniques used earlier (Jauhar et al., 2004). Spikes of DGE-1 were emasculated before anthesis, and individual florets were pollinated with pollen of 1D(1B). The two lines crossed easily without any need for hormonal treatment.

USDA-ARS, Northern Crop Science Lab., Fargo, ND 58102. Mention of trade names or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity employer. *Corresponding author (prem.jauhar@ndsu.edu).

Abbreviations: DGE, durum germplasm enhancement.

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Cytological Studies and Raising F₂ Progeny and Subsequent Generations

Mature seeds from crossed spikes were collected. F₁ seeds from each of the three crosses and selfed seed of DGE-1 and Langdon were germinated on moist filter paper in Petri dishes to determine the chromosome number from fixed root-tips stained in carbol fuchsin (Jauhar et al., 1999). The germinated seeds were planted in pots in the greenhouse. The F₁ progeny were raised and selfed to produce F₂ seeds. A total of 276 F₂ seeds derived from the DGE-1 × LDN 1D(1B) cross were planted to raise an F₂ population. Twelve seeds each of Langdon and DGE-1 were also planted to serve as controls. Spikes were fixed at various stages of development, and meiotic stages were studied in pollen mother cells in the anthers (Jauhar and Peterson, 2006; Jauhar et al., 2009). We isolated plants with 29 chromosomes that paired mostly as 13 II + 3 I. Raising three single-seed-descent generations in 2007, fertile hybrid derivatives were obtained. Based on chromosomal studies, we isolated several alien substitution lines with 28 chromosomes.

Use of Molecular Markers

Chromosome-specific markers proved useful in isolating aneuploids with a desired chromosome combination. We used *Xwmc333* (Peleg et al., 2008) for identifying chromosome 1A, *Xwgm18* (Peleg et al., 2008) for 1B, *Xwmc147* (Somers et al., 2004) for 1D, and *Xedm17* (Mullan et al., 2005) for 1E. Thus, we were able to identify homoeologous group-1 chromosomes in hybrid derivatives of the durum disomic alien addition line (Jauhar and Peterson, 2011). The segregating F₃ plants in the present study had chromosome numbers ranging from 28 to 30. Using marker analysis on these segregants, we isolated an alien disomic substitution 1E(1B) with 28 chromosomes. Seed from mature plants was harvested to raise the next generation. After confirming by cytological and molecular techniques, the desired durum alien disomic substitution line 1E(1B) with 28 chromosomes was isolated. This substitution line is cytologically stable and fertile.

Characteristics

In the durum disomic substitution 1E(1B), called DGE-3, the chromosome 1B pair of durum is replaced by a 1E pair from *L. elongatum*. The substitution line is stable and fertile and forms 14 bivalents that consist of 13 durum bivalents and one *L. elongatum* bivalent. DGE-3 is similar to its parental cultivar Langdon in plant and spike morphology (Fig. 1A), showing that the alien chromosome 1E compensates for the absence of 1B. The seeds are also very similar except that the DGE-3 seeds are smaller than those of Langdon (Fig. 1B). Earlier, we produced a durum disomic substitution line 1E(1A) that is similar to the parent cultivar Langdon and released it as genetic stock DGE-2 (Jauhar and Peterson, 2012). Similar genetic compensation abilities of *Aegilops speltoides* Tausch chromosomes for homoeologous B-genome chromosomes of hexaploid wheat have been demonstrated (Friebe et al., 2011). The average plant height of DGE-3 (based on 95 plants) is 131 cm compared with 118 cm for Langdon, 109 cm for DGE-1, and 102.4 cm for DGE-2.



Figure 1. Plant and seed morphology of durum cultivar 'Langdon' and DGE-3 disomic alien substitution line 1E(1B). (A) Mature plants of Langdon (left) and DGE-3 (right). Note the close similarity between the two plants. (B) Seeds of Langdon (left) and of DGE-3 (right). The seeds are very similar except that the DGE-3 seeds are smaller than those of Langdon.

DGE-3 produces on average 7.1 tillers. Its 100-kernel weight is 2.98 g compared with 2.50 g for DGE-1, 2.74 g for DGE-2, and 3.2 g for the parental durum cultivar Langdon.

DGE-3 is a unique genetic stock that lacks chromosome 1B and thus is null for the high-molecular-weight glutenin alleles of durum. It has the single allele that Prem Jauhar designated as *Glu-E1b* (see McIntosh et al., 2009, p. 13) derived from the alien chromosome 1E of *L. elongatum*. This genetic stock may prove useful in studies on nutritional and baking quality in durum wheat. DGE-3 may also facilitate research on relationships among the homoeologous group-1 chromosomes.

Availability

The USDA-ARS is releasing the alien substitution line for use by other researchers. Requests for seed should be addressed to Prem Jauhar at the USDA-ARS, Cereal Crops Research Unit, Northern Crop Science Lab, Fargo, ND 58102–2765, USA. Approximately 25 seeds each will be supplied on request by the users. The users would be expected to acknowledge the source of this genetic stock. The seed of DGE-3 has been deposited in the National Plant Germplasm System, Fort Collins, Colorado.

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